ECM1 is an essential factor for the determination of M1 macrophage polarization in IBD in response to LPS stimulation

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Inflammatory bowel disease (IBD) comprises chronic relapsing disorders of the gastrointestinal tract characterized pathologically by intestinal inflammation and epithelial injury. Here, we uncover a function of extracellular matrix protein 1 (ECM1) in promoting the pathogenesis of human and mouse IBD. ECM1 was highly expressed in macrophages, particularly tissue-infiltrated macrophages under inflammatory conditions, and ECM1 expression was significantly induced during IBD progression. The macrophage-specific knockout of ECM1 resulted in increased arginase 1 (ARG1) expression and impaired polarization into the M1 macrophage phenotype after lipopolysaccharide (LPS) treatment. A mechanistic study showed that ECM1 can regulate M1 macrophage polarization through the granulocyte-macrophage colony-stimulating factor/STAT5 signaling pathway. Pathological changes in mice with dextran sodium sulfate-induced IBD were alleviated by the specific knockout of the *ECM1* gene in macrophages. Taken together, our findings show that ECM1 has an important function in promoting M1 macrophage polarization, which is critical for controlling inflammation and tissue repair in the intestine.

IBD | ECM1 | GM-CSF | STAT5 | ARG1

Inflammatory bowel disease (IBD), which comprises a group of inflammatory conditions of the colon and small intestine (Crohn's disease [CD] and ulcerative colitis [UC]), can be debilitating and sometimes leads to life-threatening complications. Both genetic and environmental factors play major roles in the pathogenesis of IBD, and many IBD susceptibility loci involved in the pathogenesis of IBD have been discovered. For instance, genome-wide association studies identified NOD2 as a CD susceptibility gene, and studies have shown that NOD2 is an intracellular pattern recognition receptor that can recognize bacterial molecules and stimulate an immune reaction (1). Many susceptibility genes have also been developed as drug targets for IBD treatment. *IL23R* and *IL12* have been discovered as susceptibility genes in IBD. Both the IL23 and IL12 pathways have received much attention during the development of drugs for immune diseases, such as IBD. The human monoclonal IgG antibodies ustekinumab and MEDI2070 have been developed to block the receptor of the p40 subunit of the IL12/23 complex on leukocytes (2). However, the precise etiology of IBD remains unclear, and at present, IBD cannot be completely cured clinically.

Among the identified IBD susceptibility genes, extracellular matrix protein-1 (*ECM1*) was found to be strongly related to UC in 2008 (3). ECM1 is a multifunctional protein involved in various diseases. For example, in bone formation, ECM1 can inhibit alkaline phosphatase activity and bone mineralization to negatively regulate endochondral bone formation (4), and in skin homeostasis, loss of ECM1 function resulting from a loss-of-function mutation and the application of a serum autoantibody for ECM1 might cause lipoid proteinosis (5) and lichen sclerosus (6), respectively. ECM1 has also been associated with some types of cancer. In breast cancer, ECM1 is essential for the ability of breast cancer cells to undergo matrix attachment, invasion, and metastasis by promoting mosin membrane translocation and phosphorylation (7). In gastric cancer, ECM1 can regulate gastric cancer cell metastasis and glucose metabolism by inducing the ITGB4/FAK/SOX2/HIF-1α signal pathway (8). In 2011 and 2016, our laboratory reported the disease-related functions of ECM1 in Th2 and Th17 cells, which play major roles in asthma and multiple sclerosis, respectively (9, 10), and in 2018, our laboratory found that ECM1-deficient mice exhibit impaired TFH cell

Significance

Inflammatory bowel disease (IBD) can be debilitating, and sometimes leads to life-threatening complications. Macrophages play a critical role in colitis by secreting many cytokines and regulating tissue repair. Here, we provide evidence showing that the IBD susceptibility gene *ECM1* is critical for controlling colonic inflammation through macrophages in colitis. *ECM1* deficiency in macrophages resulted in reduced sensitivity to dextran sodium sulphate-induced colitis with increased expression of granulocyte-macrophage colony-stimulating factor (GM-CSF)-promoted arginase 1 and impaired M1 polarization by activating the STAT5 pathway. These results reveal a function of the IBD susceptibility gene *ECM1* in colonic macrophages through GM-CSF/STAT5 regulatory axis and indicate that the attenuation of ECM1 function in macrophages is a potential strategy for IBD therapy.

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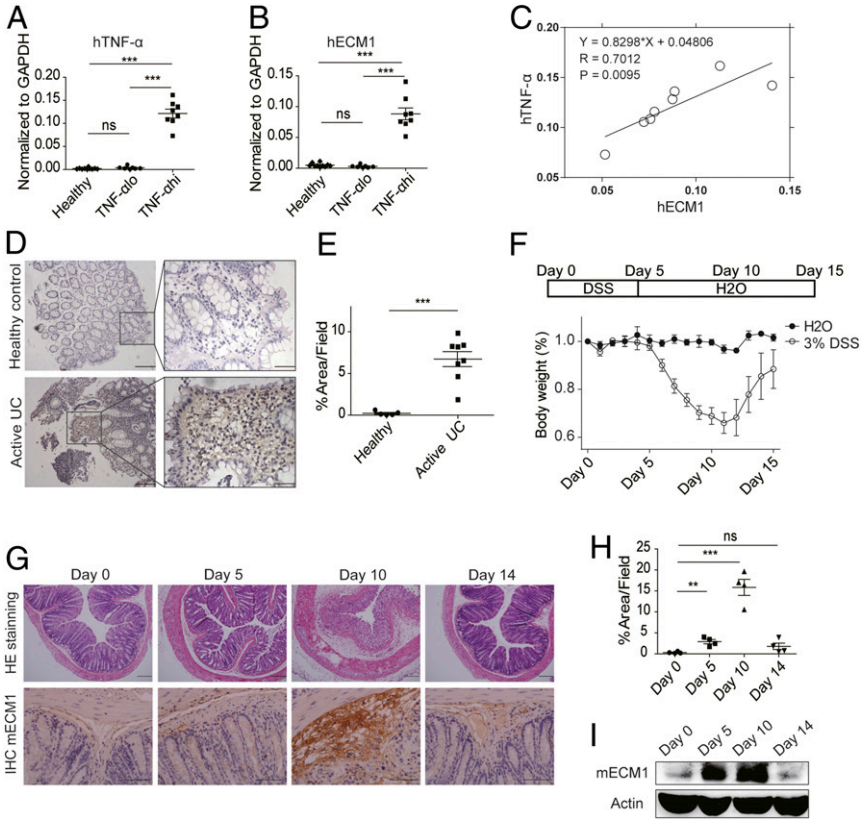

Fig. 1. Ecm1 expression is strongly correlated with the pathogenic process of IBD in both humans and mice. (*A* and *B*) Real-time quantitative PCR analysis of TNF-α and ECM1 expression in colonic mucosal tissue samples from healthy subjects ($n = 10$) and patients with inactive (TNF-αlo, $n = 7$) and active (TNF-αhi, $n = 8$) ulcerative colitis. GAPDH served as an internal reference. Different groups are indicated as diamonds (healthy individuals), circles (UC patients with low TNF-α), or squares (UC patients with low TNF-α). ***$P < 0.001$; not significant (ns), $P > 0.05$ (one-way ANOVA with Tukey's multiple comparisons test). (*C*) Analysis of the correlation between ECM1 and TNF-α gene expression ($n = 8$). Each circle indicates a tissue sample from one individual. The correlation was assessed with Pearson's test. (*D*) IHC staining of human colonic mucosal tissue sections from healthy (*Top*) subjects and patients with active UC (*Bottom*), using an anti-ECM1 antibody. *Right* is from the enlarged *Left*. (Scale bars: *Left*, 400 μm; *Right*, 100 μm.) (*E*) Quantification of ECM1 expression in the samples from healthy subjects ($n = 5$) and patients with active UC ($n = 8$) in *D*. Different groups are indicated as circles (healthy individuals) or squares (active UC patients). ***$P < 0.001$ (Student's *t* test) (*F*) Diagram (*Top*) showing the process of establishing a DSS-induced IBD mouse model. The percentage change in body weight (*Bottom*) in DSS-induced IBD mice ($n = 5$) is shown. (*G*) H&E staining (*Top*) and IHC staining with an anti-ECM1 antibody (*Bottom*) of mouse colon samples with DSS-induced IBD at different time. (Scale bars: *Top*, 200 μm; *Bottom*, 100 μm.) The data are representative of three independent experiments. (*H*) Quantification of ECM1 expression in the DSS-induced IBD mouse colon tissue samples collected at different points in *G* ($n = 4$). Different groups are indicated as circles (Day 0), squares (Day 5), up triangle (Day 10), or down triangle (Day 14). **$P < 0.01$; ***$P < 0.001$; ns, $P > 0.05$ (one-way ANOVA with Tukey's multiple comparisons test). (*I*) Immunoblot analysis of total protein extracts from DSS-induced IBD mouse colon tissue samples harvested at different points. The data are representative of three independent experiments. Actin served as a loading control throughout these experiments.

development in germinal centers (11). In addition, we previously reported that ECM1 produced by hepatocytes can prevent fibrogenesis in the mouse liver by inhibiting transforming growth factor β activation and thereby hepatic stellate cell activation (12). However, no available evidence suggests the direct function of ECM1 in IBD.

Granulocyte-macrophage colony-stimulating factor (GM-CSF), also known as colony-stimulating factor 2 (CSF2), was originally identified for its role in hematopoiesis. GM-CSF can stimulate stem cells to differentiate into granulocytes (neutrophils, eosinophils, and basophils) and monocytes (13). GM-CSF reportedly plays two different roles in inflammation. In multiple sclerosis, the blockage of GM-CSF might reduce inflammation or damage (14). However, GM-CSF has an inverse function in IBD (15). The deletion of GM-CSF in mice leads to the development of relatively severe colitis, and the administration of GM-CSF can result in clinical improvement in both mouse colitis animal models (16, 17) and patients with IBD (18). GM-CSF expression can be induced by lipopolysaccharide (LPS) stimulation (19), and GM-CSF–deficient mice are hyporesponsive to LPS (20). GM-CSF treatment also decreases the expression of proinflammatory cytokines (17). These findings indicate that GM-CSF plays critical roles in the negative regulation of inflammatory responses in the intestine after Toll-like receptor (TLR) activation. The binding of GM-CSF with its receptor is known to activate Janus kinase/signal transducer and activation of transcription (JAK/STAT) molecules. After GM-CSF activation, the cytoplasmic tail of the β common chain binds to JAKs, particularly JAK2, and the resulting complex phosphorylates and activates STAT5-A/B to induce nuclear gene transcription (13). The published data show that the activation of STAT5 by LPS is mainly dependent on GM-CSF (21). These findings suggest that the GM-CSF-induced negative regulation of TLR-mediated inflammation might rely on the activation of STAT5. However, few studies have described new molecules that are able to regulate GM-CSF/STAT5 signaling in humans.

The arginine-hydrolyzing enzyme arginase 1 (ARG1) is a central metabolic enzyme that is highly expressed and primarily located in the cytoplasm of hepatocytes (liver cells), where it catalyses the hydrolysis of arginine into urea and ornithine (22). ARG1 reportedly plays a protective role in a mouse model of colitis (23). Decreased ARG1 expression is associated with dysregulated L-arginine metabolism in UC (24), and supplementation with L-arginine can alleviate pathogenesis in dextran sulfate sodium (DSS)-induced colitis by increasing the capacity for wound repair (25). All these findings indicate protective roles for

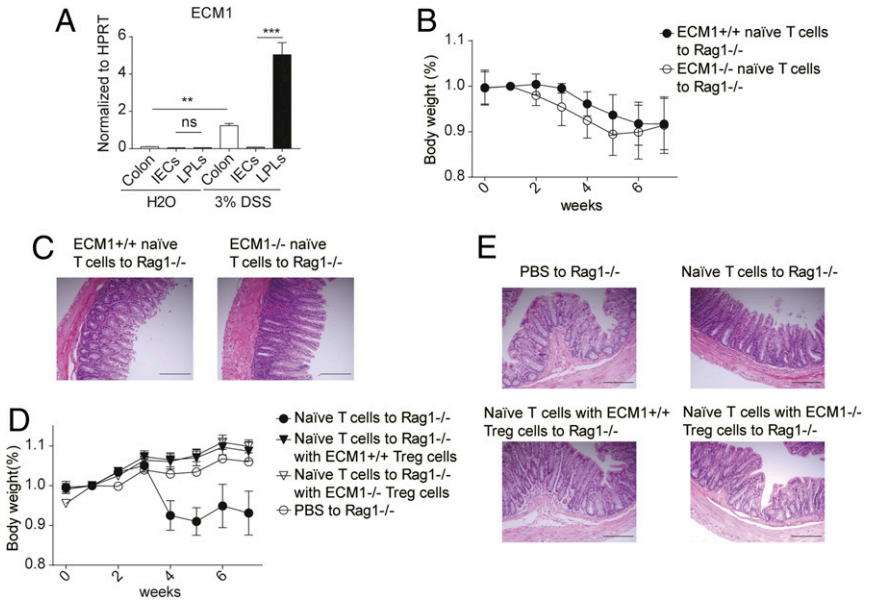

Fig. 2. ECM1 does not directly affect conventional CD4$^+$ T and Treg cell functions in the pathogenesis of IBD. (*A*) Real-time quantitative PCR analysis of ECM1 expression in colonic tissue samples, lamina propria lymphocytes (LPLs), and intestinal epithelial cells (IECs) from naive and DSS-induced IBD mice. The data are representative of three independent experiments. ***$P < 0.001$; **$P < 0.01$; not significant (ns), $P > 0.05$ (one-way ANOVA with Tukey's multiple comparisons test). (*B*) Naïve CD4$^+$ T cells from ECM1$^{+/+}$ or ECM1$^{-/-}$ mice were adoptively transferred into Rag1$^{-/-}$ mice to establish a T cell-dependent IBD mouse model. $n = 5$. (*C*) H&E staining of colon samples from the mice in *B*. (Scale bars: 200 μm.) (*D*) Mixtures of wild-type naive CD4$^+$ T cells and Treg cells from ECM1$^{+/+}$ or ECM1$^{-/-}$ mice were adoptively transferred into Rag1$^{-/-}$ mice to establish the T cell-dependent IBD mouse model. $n = 5$. (*E*) H&E staining of colon samples from the mice in *D*. (Scale bars: 200 μm.)

L-arginine metabolism and ARG1 function in colitis. The expression of ARG1 in macrophages is tightly regulated after alternative activation in M2 macrophages; therefore, ARG1 is considered a marker of M2 macrophages (26). Exposure to cytokine (IL-4 and IL-13) stimulation induces resting macrophages to activate STAT6 and thereby upregulates the mRNA and protein levels of ARG1 (27). In fact, M1 macrophages also express basal levels of ARG1, but the precise regulatory mechanism of ARG1 expression remains unknown.

In the current study, we found that ECM1 was highly expressed in macrophages from both humans and mice with IBD/colitis, and that the GM-CSF/STAT5 pathway was highly activated in ECM1-deleted macrophages after LPS-induced polarization. Furthermore, the phosphorylation of STAT5 activated by autocrine GM-CSF after LPS stimulation in macrophages was critical for the expression of ARG1. Interestingly, the deletion of ECM1 in macrophages could restrain the pathology of IBD in mice, and this effect was associated with increased ARG1 expression and M2 macrophages in the colon tissue, which indicated that ECM1 has a proinflammatory function in promoting IBD development. Our study demonstrates a function for ECM1 in promoting M1 macrophage development during IBD progression and reveals that GM-CSF/STAT5 regulation plays an important role in macrophage function after TLR stimulation.

Results

ECM1 Is Highly Expressed in Intestinal Samples from Human Patients with UC and DSS-Induced IBD Mouse Colon Tissue. Although a genome-wide association study revealed a strong correlation between ECM1 and IBD, no experimental data support this relationship (3). To confirm this association, we detected the expression of ECM1 in both human UC, which is one of the common human forms of IBD and frequently develops in the lining of the colon and rectum, and a mouse model of IBD induced by DSS. Colonic mucosal tissue samples were collected from healthy individuals ($n = 10$) and patients with UC ($n = 15$). Because cytokines have been proven to directly participate in the pathogenesis of IBD (28) and tumor necrosis factor (TNF) is considered the key functional cytokine, we selected TNF as the marker of IBD severity because TNF blockade is currently used as a standard therapy for IBD in the clinic (29). Based on the transcript level of TNF-α, we divided the patients with UC into two groups with different degrees of inflammation (i.e., a low TNF-α level group and a high-level group, named the TNF-αlo and TNF-αhi groups, respectively). Interestingly, ECM1 was highly expressed only in the TNF-αhi group, which suggested that ECM1 expression was highly induced during activation of the immune response in patients with UC (Fig. 1 *A* and *B*). Furthermore, the clinical analysis showed that the expression of ECM1 was strongly correlated with that of TNF-α (Fig. 1*C*). Immunohistochemical staining revealed that ECM1 protein expression was higher in the intestinal mucosal tissue of the patients with active UC than in that of the healthy subjects, and a histopathological analysis showed that the intestinal mucosal tissue structure was clearly destroyed in the samples from the patients with active UC (Fig. 1 *D* and *E*). To confirm this correlation between ECM1 and IBD, we established an acute IBD animal model in mice through the administration of DSS (Fig. 1*F*). Immunohistochemical staining and immunoblot analysis showed that the expression of ECM1 in colon tissue was strictly positively correlated with the pathogenic process of DSS-induced acute IBD and reached a peak on day 10 after DSS exposure (Fig. 1 *G*–*I*). Together, the data indicate that the ECM1 protein is highly correlated with the inflammatory response in both mouse and human colitis and might be involved in IBD progression.

ECM1 Does Not Directly Affect Conventional CD4$^+$ T or Treg Cell Function in the Pathogenesis of IBD. Because many immune and nonimmune cells are involved in the pathologic process of colitis, we first attempted to identify which types of cells are the main producers of ECM1. Both immune and nonimmune cells were isolated from the colon tissue of naive and DSS-IBD mice (*SI*

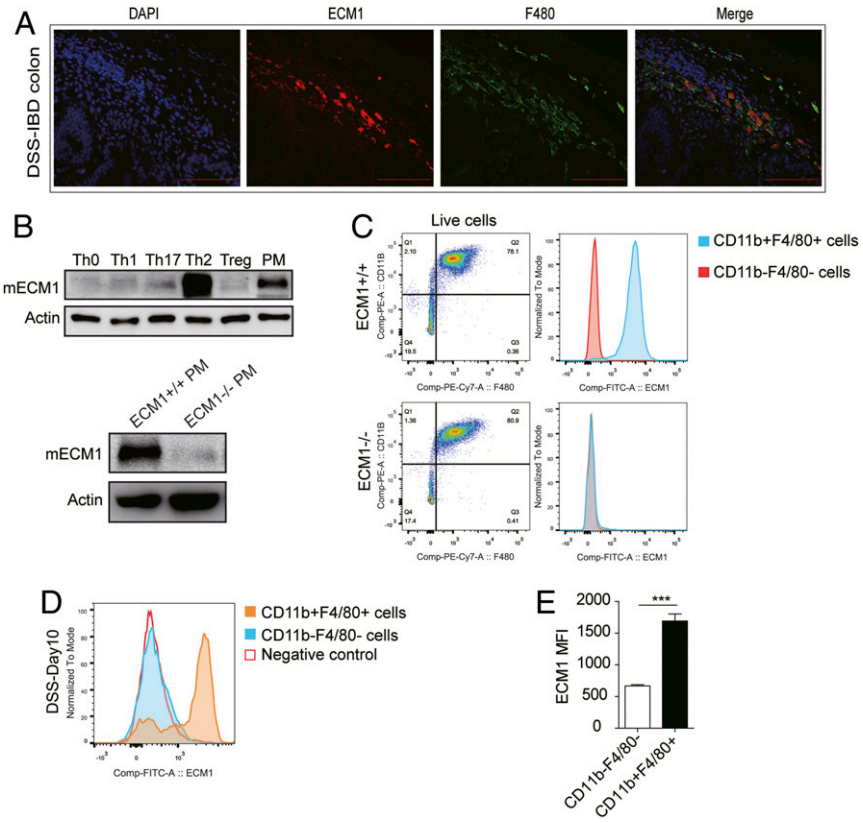

Fig. 3. ECM1 is mainly produced by macrophages in DSS-induced IBD. (*A*) Immunofluorescence analysis of ECM1 and F4/80 expression in colon tissue from DSS-induced IBD mice. (Scale bars: 100 μm.) ECM1 and F4/80 are shown in green and red, respectively. Nuclei were stained with DAPI and are shown in blue. (*B*) Immunoblot analysis of ECM1 in $CD4^+$ helper T cell subsets after in vitro differentiation and in macrophages isolated from the peritoneal cavity (PM: peritoneal macrophage). Actin served as a loading control throughout these experiments. The data are representative of three independent experiments. Flow cytometric analysis of ECM1 in macrophages ($CD11B^+F4/80^+$) and nonmacrophage cells ($CD11B^-F4/80^-$) isolated from the peritoneal cavity of $ECM1^{+/+}$ and $ECM1^{-/-}$ mice (*C*) and the lamina propria of $ECM1^{+/+}$ mice with DSS-induced colitis on day 10 (*D*). The data are representative of three independent experiments. (*E*) Quantification of the ECM1 mean fluorescence intensity in *D* ($n = 4$). ***$P < 0.001$ (Student's *t* test).

Appendix, Fig. S1 *A* and *B*). Quantitative real-time PCR showed low ECM1 expression in the colon of the naive mice, but ECM1 expression was significantly up-regulated in the colon and lamina propria lymphocytes after DSS treatment. However, under the same conditions, ECM1 expression was not detected in intestinal epithelial cells (Fig. 2*A*). IBD is considered a T cell-mediated disease, and $CD4^+$ T cells, particularly Treg cells, are vitally important in the orchestration of excessive inflammation (30, 31). Our published studies have also shown that ECM1 functions in $CD4^+$ T cells (9–11). Therefore, we first validated the functions of ECM1 in $CD4^+$ T cells in IBD. The T cell transfer model of colitis is the most compelling design for studying the functions of $CD4^+$ T and Treg cells in IBD. In this model, Rag1-knockout mice were peritoneally injected with wild-type or ECM1-knockout naive $CD4^+$ T cells, and their body weight was measured weekly for 7 wk. The data showed that ECM1 was not responsible for naïve $CD4^+$ T cell-mediated colitis (Fig. 2 *B* and *C*). The experiments studying the transfer of splenic Treg cells mixed with wild-type naive $CD4^+$ T cells into Rag1-knockout mice revealed that splenic Treg cells from ECM1-knockout mice could still inhibit colitis (Fig. 2 *D* and *E*). These data suggest that ECM1 derived from conventional $CD4^+$ T and Treg cells does not play an important role in IBD induction.

ECM1 Is Mainly Produced by Macrophages in DSS-Induced IBD. Because lamina propria lymphocytes are the major cell type that produces ECM1, we searched the Immunological Genome Project (http://www.immgen.org/) online database for ECM1, and all the information suggests that ECM1 is highly expressed in peritoneal macrophages. To address this possibility, the expression of ECM1 in macrophages was analyzed. Immunofluorescence staining showed that ECM1 colocalized with macrophages labeled with an antibody specific for F4/80 in the mouse colon after DSS treatment (Fig. 3*A*). To further confirm the expression of ECM1 in macrophages, an immunoblot analysis was performed to detect the expression of ECM1 in peritoneal macrophages from wild-type and ECM1-knockout mice and in vitro-differentiated $CD4^+$ T cell subsets, particularly Th2 cells, which were used as a positive control. The results showed that ECM1 was indeed highly expressed in Th2 cells, but not in the other CD4 subsets, as previously reported (9). In addition, high expression of ECM1 was also detected in peritoneal macrophages (Fig. 3*B*). A flow cytometric analysis showed that ECM1 was expressed only in macrophages (Fig. 3*C*), particularly those harvested from pathogenetic colon tissue after IBD induction (Fig. 3 *D* and *E* and *SI Appendix*, Fig. S2*B*), but not in other cells from the peritoneal cavity (*SI Appendix*, Fig. S2*A*). In addition, the human monocyte-macrophage cell line THP1 can differentiate from suspended monocytes into adherent macrophages with increased expression of ECM1 (*SI Appendix*, Fig. S2 *C* and *D*). Human primary macrophages differentiated from peripheral blood mononuclear cells collected from healthy subjects could also express high ECM1 levels (*SI Appendix*, Fig. S2*E*). It is widely accepted that macrophages consist of two populations in vivo: the tissue-resident macrophage population established before birth and self-maintained during adulthood

and the population differentiated from bone marrow-derived blood monocytes (32, 33). To identify which population of macrophages is the main population expressing ECM1, we transferred bone marrow from wild-type and ECM1-knockout mice (CD45.2 background) into lethally irradiated wild-type CD45.1-background mice. After 8 wk of reconstitution, a flow cytometric analysis showed high-efficiency reconstitution (*SI Appendix*, Fig. S2*F*) and indicated that ECM1 might be highly expressed in both tissue-resident macrophages and bone marrow-derived macrophages (BMMs; *SI Appendix*, Fig. S2*G*). Therefore, the above data indicate that ECM1 is highly expressed in mouse and human macrophages, particularly macrophages from inflamed colon tissue in IBD.

Macrophages from ECM1-Deficient Mice Exhibit Enhanced M2 Polarization and Impaired Proinflammatory Function. To study the function of ECM1 in macrophages, we generated conditional ECM1-knockout mice (Lyz-Cre/ECM1 f/f) (*SI Appendix*, Fig. S3*A*); ECM1-knockout ($ECM1^{-/-}$) mice did not survive past the age of 6 to 8 wk because they developed spontaneous organ lesions in the liver (12). Although Lyz-Cre mice show specific Cre expression in both macrophages and neutrophils, a flow cytometric analysis showed that ECM1 was not expressed in neutrophils, mast cells, or basophils (*SI Appendix*, Fig. S2*A*). Thus, Lyz-Cre/ECM1 f/f mice show deficiency in the *ECM1* gene, specifically in the macrophage lineage. Flow cytometric analysis, quantitative PCR, and immunoblot analyses all confirmed the deletion of ECM1 in macrophages (*SI Appendix*, Fig. S3 *B–D*). Characterization of the steady-state immune cell populations revealed normal immune homeostasis in the spleen, lymph nodes, and thymus of Lyz-Cre/ECM1 f/f mice compared with those of their wild-type ECM1 f/f littermates (*SI Appendix*, Fig. S3*E*). An analysis of RNA-sequencing data indicated an alternative metabolic process and a higher arginine metabolic level in Lyz-Cre/ECM1 f/f compared with ECM1 f/f macrophages (Fig. 4 *A* and *B*). L-arginine metabolism has been recognized to be involved in macrophage functions, and ARG1 is regarded as a functional marker for alternatively activated macrophages, also termed M2 macrophages (26, 34, 35). We further confirmed that ARG1 expression was significantly up-regulated after LPS simulation in peritoneal macrophages from Lyz-Cre/ECM1 f/f mice compared with those from ECM1 f/f macrophages (Fig. 4*C*). ARG1 reportedly suppresses intestinal inflammation (36, 37). Further analysis showed that the CD206 expression level in Lyz-Cre/ECM1 f/f macrophages was higher than that in ECM1 f/f macrophages (Fig. 4 *D* and *E*), which suggested the development of an M2 macrophage population. In addition, the proinflammatory cytokines TNF-α and IL-6 were also reduced in Lyz-Cre/ECM1 f/f macrophages (Fig. 4*F*). The above-described observations indicate that macrophages from ECM1-deficient mice exhibit an impaired M1 phenotype.

Autocrine GM-CSF Is Responsible for the Enhanced Expression of ARG1 in ECM-1-Deficient Macrophages. M1 macrophages are classically activated by IFN-γ or LPS, and M2 macrophages are alternatively activated by IL-4, IL-10, or IL-13. We first selected LPS and IL-4 for M1- and M2-polarizing stimulation, respectively. Interestingly, ARG1 expression was significantly enhanced in Lyz-Cre/ECM1 f/f macrophages compared with ECM1 f/f macrophages after LPS stimulation, but ARG1 expression in Lyz-Cre/ECM1 f/f and ECM1 f/f macrophages remained similar after IL-4 stimulation, which suggested that under inflammatory conditions, ECM1 can inhibit LPS-mediated ARG1 expression in the intestine (Fig. 5*A*).

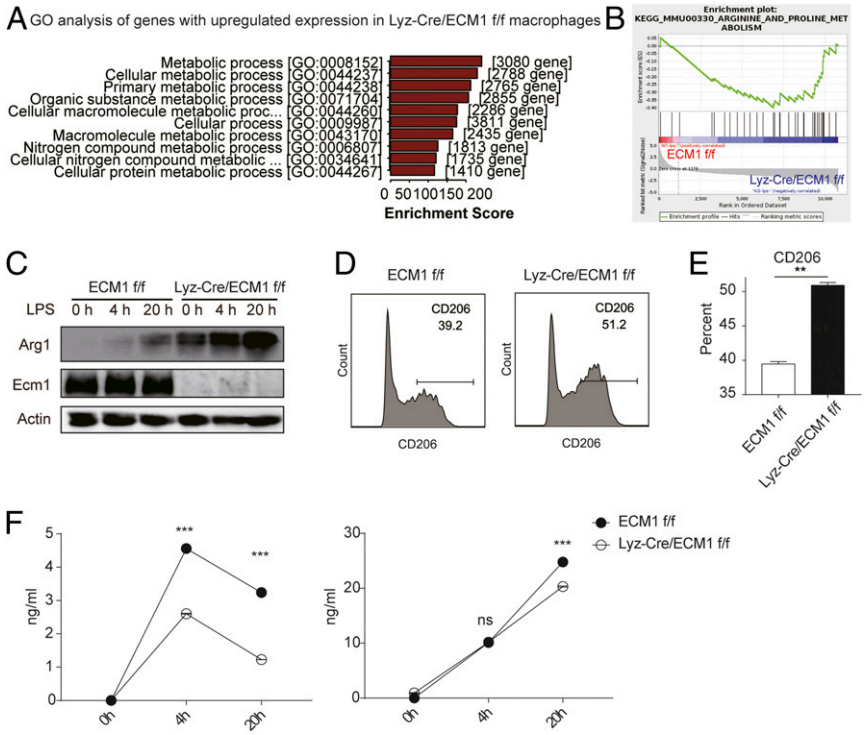

Fig. 4. Macrophages from ECM1-deficient mice exhibit enhanced M2 polarization and impaired proinflammatory function. (*A*) GO analysis of genes showing up-regulated expression in Lyz-Cre/ECM1 f/f macrophages, as demonstrated in the RNA-Seq data. (*B*) GSEA analysis of arginine and proline metabolism-related genes in ECM1 f/f and Lyz-Cre/ECM1 f/f macrophages. (*C*) Immunoblot analysis of ECM1 and ARG1 expression in peritoneal macrophages stimulated with LPS for 0, 4, and 20 h. The data are representative of three independent experiments. Actin served as a loading control throughout this experiment. (*D*) Flow cytometric analysis of CD206 in peritoneal macrophages from ECM1 f/f and Lyz-Cre/ECM1 f/f mice. The data are representative of three independent experiments. (*E*) Quantification of the percentage of $CD206^+$ cells in *D* ($n = 3$). $^{**}P < 0.01$ (Student's *t* test). (*F*) ELISA of the levels of TNF-α and IL-6 secreted by ECM1 f/f and Lyz-Cre/ECM1 f/f peritoneal macrophages stimulated with LPS for 0, 4, and 20 h. $^{***}P < 0.001$; not significant (ns), $P > 0.05$ (Student's *t* test). The data are representative of three independent experiments.

It has been reported that both GM-CSF and M-CSF are able to drive macrophage differentiation from bone marrow cells (BMMs). The analysis of stimulation with LPS and/or IFN-γ revealed that LPS activation was a strong factor for ARG1 expression only in GM-CSF–differentiated BMMs and not in M-CSF-differentiated BMMs (Fig. 5*B*). Because LPS activation can promote GM-CSF secretion (21), we found that GM-CSF production was higher in Lyz-Cre/ECM1 f/f macrophages than in ECM1 f/f macrophages after LPS activation (Fig. 5*C*). To further confirm whether GM-CSF plays a determining role, we treated peritoneal macrophages with an anti–GM-CSF neutralizing antibody before LPS activation. As expected, GM-CSF depletion abolished the enhancement in ARG1 expression in Lyz-Cre/ECM1 f/f macrophages compared with ECM1 f/f macrophages (Fig. 5*D*). Furthermore, GM-CSF depletion reduced autocrine GM-CSF production (Fig. 5*E*). An anti-CD25 antibody could not impair ARG1 and GM-CSF production and was considered a negative control. Both GM-CSF and LPS were able to regulate ARG1 expression, but played different regulatory roles on IL-6 and TNF-α expression (Fig. 5*F*). After GM-CSF stimulation, no difference in ARG1 expression was found between ECM1 f/f and Lyz-Cre/ECM1 f/f macrophages (Fig. 5*G*). Based on the above-described observations, we assumed that the LPS/GM-CSF/ARG1 axis plays an important role in regulating macrophage function. The deletion of ECM1 in macrophages significantly enhances the LPS-stimulated production of ARG1, and this process relies on the production of GM-CSF.

ECM1 Inhibits ARG1 Expression through the GM-CSF/STAT5 Pathway during M1 Macrophage Polarization. We subsequently explored the mechanism regulating ARG1 expression in Lyz-Cre/ECM1 f/f macrophages. An immunoblot analysis showed no significant differences in the activation of NF-κB or MAPKs (Jnk/Erk/P38), the PI3K/Akt signaling pathway, or the STAT3/6 signaling pathway between ECM1 f/f and Lyz-Cre/ECM1 f/f macrophages after LPS stimulation (Fig. 6*A* and *SI Appendix*, Fig. S4*A*). This finding indicated nonclassical regulation after LPS activation. Further analysis revealed that the phosphorylation of STAT5 in ECM1-deficient macrophages was stronger than that in wild-type macrophages. Furthermore, anti–GM-CSF neutralizing antibody

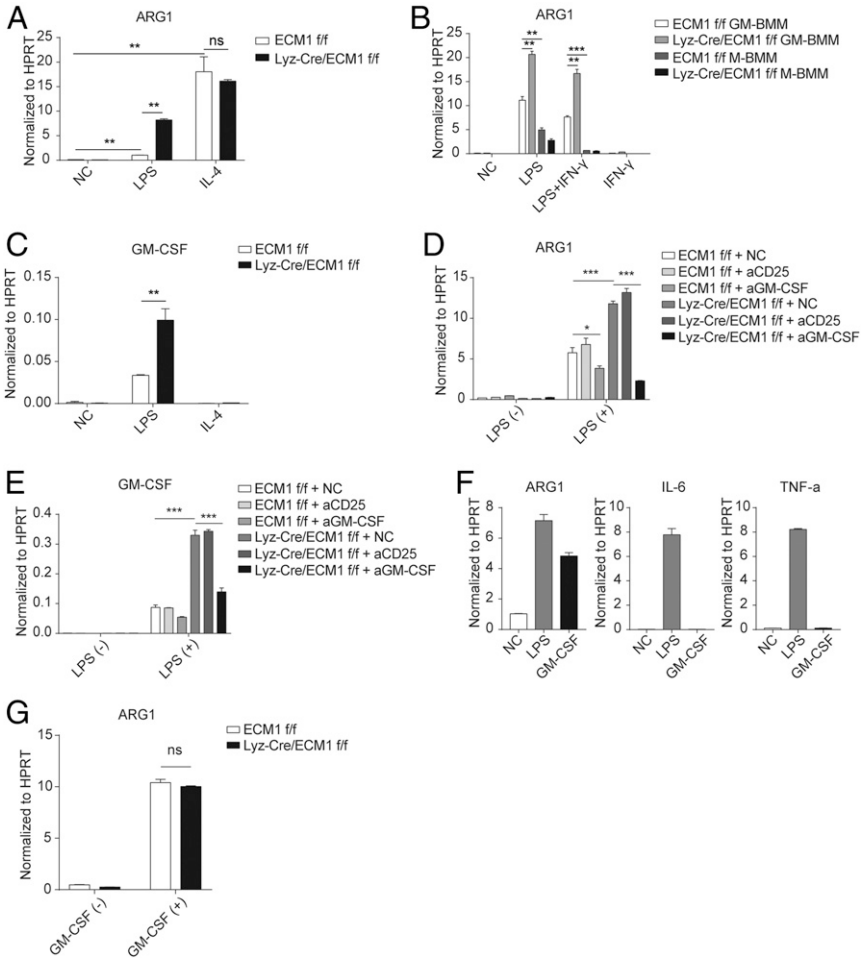

Fig. 5. Autocrine GM-CSF is responsible for enhanced arginase 1 expression in ECM1-deficient macrophages. (*A*) Quantitative analysis of ARG1 expression in ECM1 f/f and Lyz-Cre/ECM1 f/f peritoneal macrophages stimulated with LPS (200 ng/mL) or IL-4 (10 ng/mL). $^{**}P < 0.01$; not significant (ns), $P > 0.05$ (Student's *t* test). (*B*) Quantitative analysis of ARG1 expression in GM-CSF-differentiated BMMs and M-CSF-differentiated BMMs stimulated with LPS (200 ng/mL) and/or IFN-γ (50 ng/mL). $^{**}P < 0.01$; $^{***}P < 0.001$ (Student's *t* test). (*C*) Quantitative analysis of GM-CSF expression in ECM1 f/f and Lyz-Cre/ECM1 f/f peritoneal macrophages stimulated with LPS (200 ng/mL) or IL-4 (10 ng/mL). $^{**}P < 0.01$ (Student's *t* test). Quantitative analysis of the ARG1 (*D*) and GM-CSF (*E*) levels in ECM1 f/f and Lyz-Cre/ECM1 f/f peritoneal macrophages treated with anti-CD25 antibody (10 μg/mL) or anti–GM-CSF antibody (10 μg/mL) and then stimulated with LPS. $^{***}P < 0.001$; $^{*}P < 0.05$ (Student's *t* test). (*F*) Quantitative analysis of the ARG1, IL-6, and TNF-α levels in peritoneal macrophages stimulated with LPS (200 ng/mL) or GM-CSF (10 ng/mL). (*G*) Quantitative analysis of ARG1 expression in ECM1 f/f and Lyz-Cre/ECM1 f/f peritoneal macrophages stimulated with GM-CSF (10 ng/mL). ns, $P > 0.05$. All the cells were stimulated for 20 h, and all the data are representative of three independent experiments.

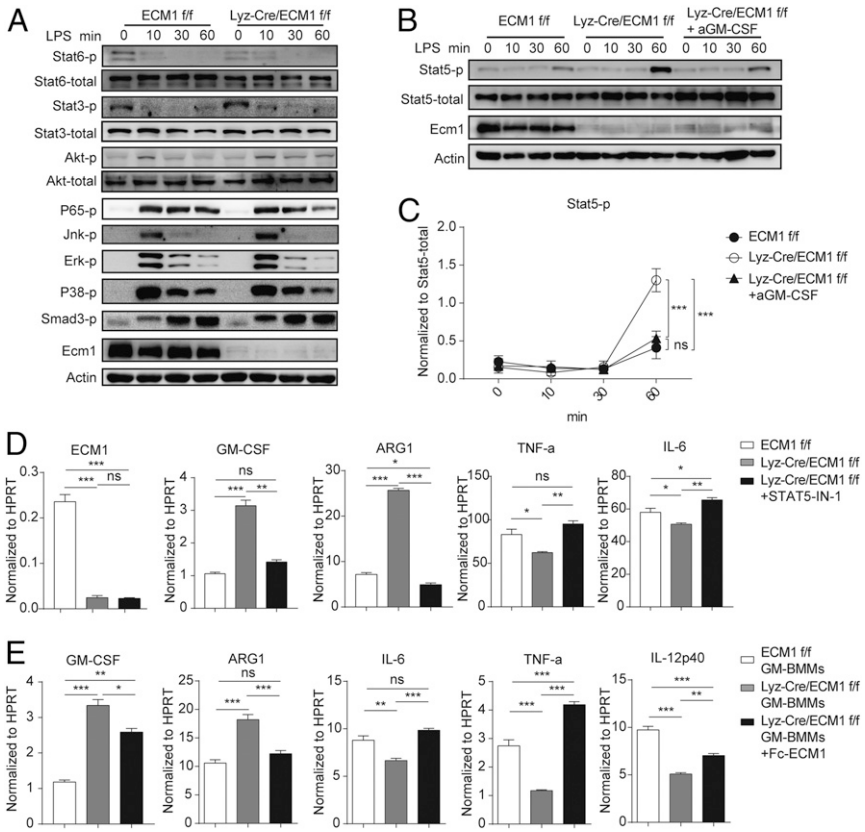

Fig. 6. ECM1 inhibits ARG1 expression through the GM-CSF/STAT5 pathway during M1 macrophage polarization. (*A*) Immunoblot analysis of STAT3/6, NF-κB, MAPK (Jnk/Erk/P38), and PI3K/Akt signaling activation in ECM1 f/f and Lyz-Cre/ECM1 f/f peritoneal macrophages stimulated with LPS. (*B*) Immunoblot analysis of STAT5 signaling activation in ECM1 f/f and Lyz-Cre/ECM1 f/f peritoneal macrophages treated with or without anti–GM-CSF antibody and then stimulated with LPS. (*C*) Quantification of the results from the Western blot analysis of STAT5 activation (phosphorylated STAT5/total STAT5) in *B*. ***$P < 0.001$; not significant (ns), $P > 0.05$ (Student's *t* test). (*D*) Quantitative PCR analysis of ECM1, ARG1, GM-CSF, TNF-α, and IL-6 expression in ECM1 f/f and Lyz-Cre/ECM1 f/f peritoneal macrophages treated with DMSO/STAT5-IN-1 for 3 h and then stimulated with LPS for 20 h. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns, $P > 0.05$ (Student's *t* test). (*E*) Quantitative PCR analysis of GM-CSF, ARG1, IL-6, IL-12p40, and TNF-α expression in ECM1 f/f and Lyz-Cre/ECM1 f/f GM-CSF-differentiated BMMs treated with recombinant Fc-ECM1 protein (20 μg/mL) for 7 d and then stimulated with LPS for 20 h. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns, $P > 0.05$ (one-way ANOVA with Tukey's multiple comparisons test). All the data are representative of three independent experiments.

reduced the phosphorylation of STAT5 (Fig. 6 *B* and *C*). To study the function of STAT5 activation, we selected the STAT5 inhibitor STAT5-IN-1 to reduce the phosphorylation of STAT5 without affecting other signaling pathways (*SI Appendix*, Fig. S4*B*). Quantitative real-time PCR showed that STAT5-IN-1 was able to inhibit the LPS-induced expression of ARG1 and GM-CSF (*SI Appendix*, Fig. S4*C*). In ECM1-deficient macrophages, STAT5-IN-1 strongly reduced GM-CSF/ARG1 expression and rescued cytokine production (Fig. 6*D*). To confirm the direct function of ECM1, we found that recombinant protein Fc-ECM1 could inhibit STAT5 phosphorylation (*SI Appendix*, Fig. S4*D*) and rescue the phenotype of Lyz-Cre/ECM1 f/f macrophages by downregulating the expression of GM-CSF/ARG1 and upregulating the expression of inflammatory cytokines (IL-6, IL-12p40, and TNF-α; Fig. 6*E*). GM-CSF stimulation did not strengthen STAT5 phosphorylation in ECM1-deficient macrophages at different times and doses, which suggested that autocrine GM-CSF production might be critical for determination of the Lyz-Cre/ECM1 f/f macrophage phenotype (*SI Appendix*, Fig. S4 *E* and *F*). Although there was protective function described previously for ARG1 and L-arginine metabolism in colitis, addition of an ARG-1 inhibitor (*SI Appendix*, Fig. S4*G*) or excess L-arginine (*SI Appendix*, Fig. S4*H*) failed to rescue proinflammatory cytokine expression from ECM1-deficient macrophages, which suggested that ARG1 expression in ECM1-deficient macrophages might not affect macrophages themselves and synergized with impaired inflammation to alleviate colitis. All these data indicate the importance of the GM-CSF/STAT5 pathway in regulating ECM1-dependent ARG1 expression, and thereby M1 macrophage polarization and a potential immunosuppressive function of STAT5 in macrophages.

ECM1 Could Aggravate Colitis through Macrophages In Vivo. To study the in vivo function of ECM1 in macrophages in IBD, we used a DSS-induced IBD mouse model and found that ECM1 could aggravate colitis through macrophages. After 5 d of drinking DSS-supplemented water, mice acquired the pathological characteristics of IBD. However, compared with ECM1 f/f mice, Lyz-Cre/ECM1 f/f mice showed alleviated symptoms of IBD, such as an increased body weight, a lower mortality rate, and a reduced disease score (Fig. 7 *A* and *B* and *SI Appendix*, Fig. S5*A*). Analysis of representative images, the colon length, and hematoxylin and eosin (H&E) staining of colon tissue also revealed that the Lyz-Cre/ECM1 f/f mice could resist DSS-induced IBD (Fig. 7 *C*–*E*). Immunofluorescence revealed that ECM1 was specifically deleted in macrophages in DSS-induced IBD (*SI Appendix*, Fig. S5*B*). Although the number of colonic macrophages in the Lyz-Cre/ECM1 f/f mice was similar to that in the ECM1 f/f mice with colitis (Fig. 7*F*), the percentage of M2 macrophages was higher in colon tissue from the Lyz-Cre/ECM1 f/f mice than in that from the ECM1 f/f mice (Fig. 7 *G* and *H*). Further analysis showed reduced secretion of proinflammatory cytokines (IL-6 and TNF-α) and

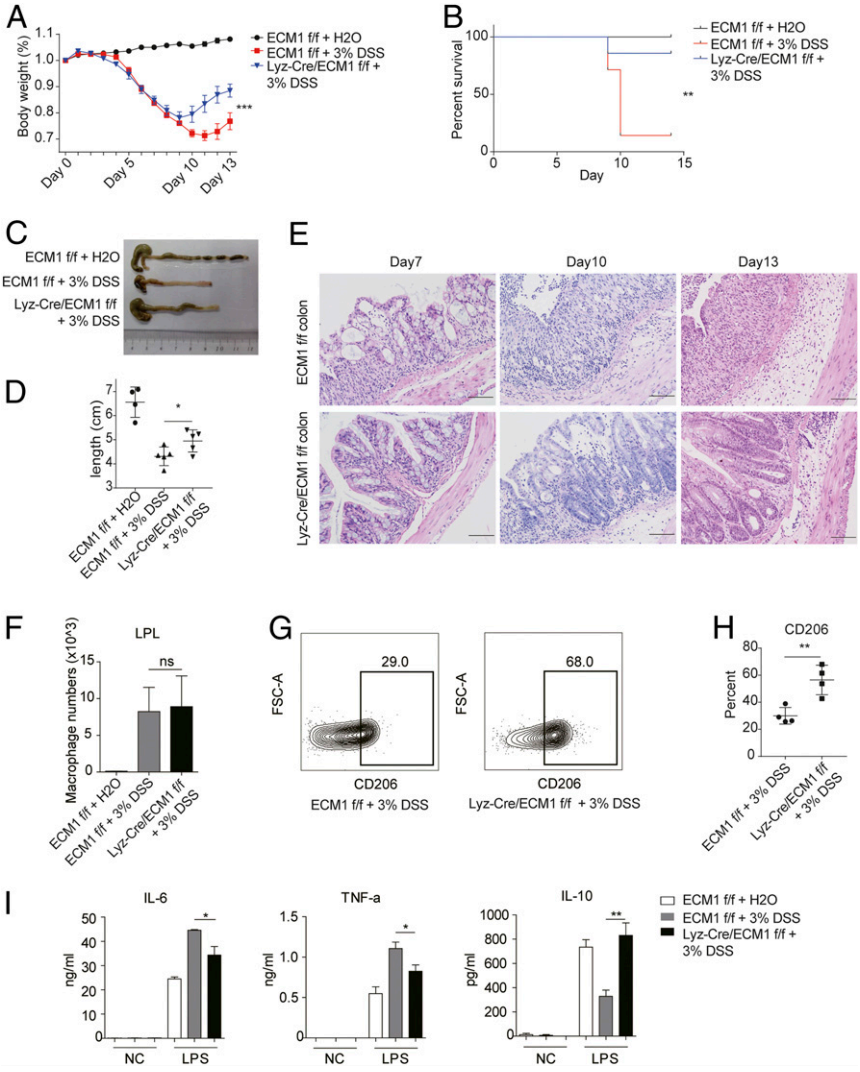

Fig. 7. Ecm1-aggravated colitis through macrophages in vivo. (*A*) Percentage changes in the body weights of ECM1 f/f and Lyz-Cre/ECM1 f/f mice with colitis. The small horizontal lines indicate the means ± SEMs. ***$P < 0.001$ (Student's *t* test). (*B*) Percentage survival of the ECM1 f/f and Lyz-Cre/ECM1 f/f mice with DSS-induced IBD in *A*. **$P < 0.01$ (Student's *t* test). Representative images (*C*) and length (*D*) of the colon (on day 13) of the ECM1 f/f and Lyz-Cre/ECM1 f/f mice with colitis in *A*. Different groups are indicated as circles (ECM1 f/f + H_2O), up triangle (ECM1 f/f + 3% DSS), or down triangle (Lyz-Cre/ECM1 f/f + 3% DSS). *$P < 0.05$ (Student's *t* test). (*E*) H&E staining of colonic tissue samples harvested on days 7, 10, and 13 from the ECM1 f/f and Lyz-Cre/ECM1 f/f mice with colitis. (Scale bars: 200 μm.) (*F*) Flow cytometric analysis of macrophage counts in the colonic lamina propria of ECM1 f/f and Lyz-Cre/ECM1 f/f mice with colitis on day 13. Not significant (ns), $P > 0.05$ (Student's *t* test). (*G*) Flow cytometric analysis of CD206 in macrophages from the colonic lamina propria of ECM1 f/f and Lyz-Cre/ECM1 f/f mice with DSS-induced colitis on day 10. (*H*) Quantification of the percentage of $CD206^+$ cells in *F*. Different groups are indicated as circles (ECM1 f/f + 3% DSS) or squares (Lyz-Cre/ECM1 f/f + 3% DSS). **$P < 0.01$ (Student's *t* test). (*I*) ELISA of the IL-6, TNF-α z, and IL-10 levels in the supernatants of peritoneal macrophages collected from ECM1 f/f and Lyz-Cre/ECM1 f/f mice with colitis after stimulation with LPS for 20 h. **$P < 0.01$; *$P < 0.05$ (Student's *t* test).

increased secretion of an anti-inflammatory cytokine (IL-10) by peritoneal macrophages from the Lyz-Cre/ECM1 f/f mice with colitis (Fig. 7*I*). Because GM-CSF-activated monocytes can protect mice from colitis through T cells (38), we also found increased numbers of suppressive T cells (Treg cells) in colon tissue (*SI Appendix*, Fig. S5*C*). In summary, during IBD induction, increased numbers of macrophages accumulated in the inflamed colon, where ECM1 was produced and consequently aggravated colitis in vivo.

Discussion

Here, we report an important function of the IBD susceptibility gene *ECM1*, which can promote the pathology of colitis through repression of the GM-CSF/STAT5 signaling pathway during macrophage polarization (Fig. 8). Our results revealed that ECM1 was highly expressed in colonic mucosal tissue samples from patients with activated inflammation and was highly correlated with the pathology of colitis in both human and mouse colitis. In the current study, we showed that in DSS-induced mouse colitis, ECM1 was identified as a macrophage-derived protein. Macrophages with ECM1 deficiency were endowed with alternative or M2 macrophage characteristics. RNA sequencing indicated enhanced arginine metabolism, and the expression of the M2 macrophage-related gene *ARG1* was increased significantly in ECM1-deficient macrophages in response to TLR-mediated activation. The analysis of the underlying mechanism revealed that GM-CSF expression was up-regulated in ECM1-deficient macrophages, and that this up-regulation was critical for TLR-mediated ARG1 expression but not for IL-4–mediated ARG1 expression. Furthermore, we analyzed the TLR-mediated signaling pathways and found that GM-CSF mediated STAT5 activation, and this activation was critical for TLR-mediated ARG1 expression and M1/2 macrophage polarization. In vivo data indicated that ECM1 deficiency in

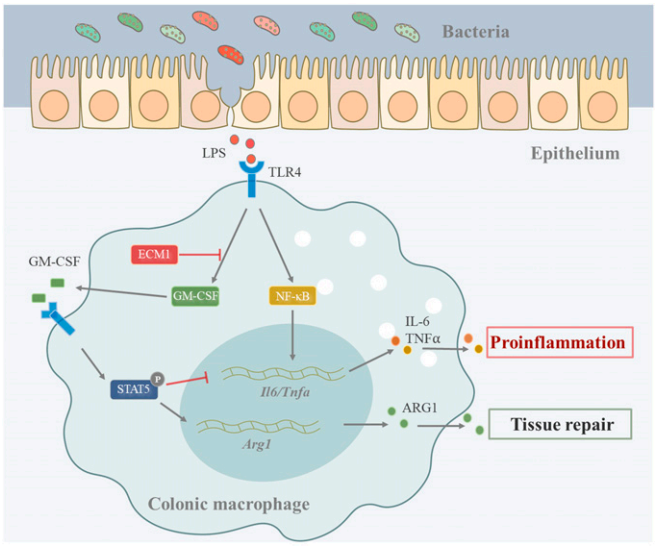

Fig. 8. Schematic of our working mechanism. During the process of colitis, a large number of macrophages migrate into the inflammatory sites of intestinal tissue and are stimulated with components (e.g., lipopolysaccharides) from the invading microbiota. Those colonic macrophages can express extracellular matrix protein 1 (ECM1), which promotes inflammation and suppresses tissue repair by inhibiting TLR-mediated granulocyte-macrophage colony-stimulating factor (GM-CSF) expression. After ECM1 deletion in macrophages, a higher amount of autocrine GM-CSF is secreted from LPS (lipopolysaccharides)-stimulated macrophages, and the secreted GM-CSF in turn activates signal transducer and activation of transcription 5 (STAT5). Phosphorylated STAT5 can suppress inflammatory cytokine transcription and promote expression of the M2 macrophage-related gene ARG1, which functions in tissue repair. Enhanced ARG1 expression and arginine metabolism synergize with impaired inflammation to alleviate colitis when ECM1 is deleted from macrophages. White circles indicate vesicles; colored circles indicate nucleus; red bacteria indicate gram-negative bacteria; green bacteria indicate gram-positive bacteria.

macrophages could alleviate the pathology of colitis by decreasing the immune response without affecting the macrophage numbers. These data suggest that the proinflammatory function of ECM1-deficient macrophages is diminished in colitis. Our study provides experimental evidence showing the function of ECM1, which was previously identified as an IBD susceptibility gene (3), in colitis. We also found GM-CSF/STAT5-mediated ARG1 regulation after TLR activation, but not IL4 activation. According to our findings, we hypothesize that patients with IBD with ECM1 SNPs or accumulated ECM1 protein in the colon may exhibit relatively low STAT5 activation and ARG1 expression, resulting in uncontrolled inflammation and aggravated disease.

Macrophages play a critical role in colitis by secreting many cytokines, and GM-CSF is an important cytokine produced by macrophages. Specifically, GM-CSF is crucial for the maturation of macrophages, dendritic cells, and granulocytes, and plays proinflammatory functions in many immune-mediated diseases, such as multiple sclerosis (14). However, GM-CSF has also been considered a therapeutic drug in IBD (15). These dual and opposite characteristics make the use of GM-CSF physiologically complicated. Before cell maturation, GM-CSF and M-CSF mainly promote cell differentiation. However, in mature cells such as macrophages, GM-CSF functions as an immune regulatory factor by activating downstream signaling pathways. GM-CSF alone can slightly increase the inflammatory response (39). The TLR-mediated secretion of GM-CSF likely cannot promote further signaling activation from that induced by TLR signaling. Therefore, the TLR-mediated secretion of GM-CSF, whose proinflammatory function might be overwhelmed after TLR activation, may relieve inflammation by impairing M1 polarization in macrophages through the JAK/STAT signaling pathway. The STAT5 signaling pathway might be the critical factor for the antiinflammatory function of GM-CSF in macrophages. The protective functions of anti–TNF-α antibody and chronic growth hormone in colitis are also related to the activation of STAT5 (40, 41). Epithelial GM-CSF/STAT5 signaling is reportedly essential for the intestinal homeostatic response to gut injury (42). Because STAT5 is a transcription factor, we hypothesize that the nuclear transcriptional regulatory function of STAT5 after GM-CSF activation leads to the expression of M2 macrophage-related genes, such as *ARG1*. This finding might explain the mechanism through which GM-CSF exerts an antiinflammatory function in IBD involving disorder in the mucosa-associated microbiota but a proinflammatory function in autoimmune diseases, such as experimental autoimmune encephalitis (43).

Materials and Methods

All data are contained in the manuscript text and *SI Appendix*. Detailed information on the materials, methods, and associated references can be found in the *SI Appendix*.

Patients. Human colonic mucosal samples were collected from healthy volunteers (5 men and 5 women, aged 30–58 y) and patients with UC (10 men and 5 women, aged 30–58 y) during endoscopy at the Department of Digestive Diseases, Huashan Hospital, Fudan University. Ethical approval was provided by the Hospital Ethics Committee (Ethics Approval 2013-005-V1).

Mice. CD45.1, Rag1$^{-/-}$ (11), ECM1$^{-/-}$ (9), and Lyz-Cre mice (44) have been described previously. ECM1 f/f mice were designed by inserting two loxP sites into the intron between the fifth and sixth exons and the intron between the eighth and ninth exons (*SI Appendix*, Fig. S3*A*) and generated in the C57BL/6 background using CRISPR/Cas9 technology. The mice were bred and housed under specific pathogen-free conditions. Age (6–12 wk)-matched and sex-matched experimental mice and littermates were used. All the animal experiments were performed in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* (45).

Statistical Analysis. The data are presented as the means ± SEMs. P values < 0.05 were considered significant. The significance of the differences was analyzed using Student's t test and one-way or two-way ANOVA, as indicated. Kaplan-Meier survival curves were tested using the log-rank (Mantel-Cox) test.

Ethics Approval. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The use of human samples was approved by the Department of Digestive Diseases, Huashan Hospital, Fudan University (Ethics Approval 2013-005-V1). These samples were deidentified before use in this study, and all participants gave informed consent.

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1. A. Negroni, M. Pierdomenico, S. Cucchiara, L. Stronati, NOD2 and inflammation: Current insights. *J. Inflamm. Res.* **11**, 49–60 (2018).
2. M. A. Raad, N. H. Chams, A. I. Sharara, New and evolving immunotherapy in inflammatory bowel disease. *Inflamm. Intest. Dis.* **1**, 85–95 (2016).
3. S. A. Fisher *et al.*; Wellcome Trust Case Control Consortium, Genetic determinants of ulcerative colitis include the ECM1 locus and five loci implicated in Crohn's disease. *Nat. Genet.* **40**, 710–712 (2008).
4. M. M. Deckers *et al.*, Recombinant human extracellular matrix protein 1 inhibits alkaline phosphatase activity and mineralization of mouse embryonic metatarsals in vitro. *Bone* **28**, 14–20 (2001).
5. T. Hamada *et al.*, Lipoid proteinosis maps to 1q21 and is caused by mutations in the extracellular matrix protein 1 gene (ECM1). *Hum. Mol. Genet.* **11**, 833–840 (2002).
6. I. Chan *et al.*, Characterization of IgG autoantibodies to extracellular matrix protein 1 in lichen sclerosus. *Clin. Exp. Dermatol.* **29**, 499–504 (2004).

7. Q. Wu *et al*., Extracellular matrix protein 1 recruits moesin to facilitate invadopodia formation and breast cancer metastasis. *Cancer Lett.* **437**, 44–55 (2018).
8. L. Gan *et al*., Extracellular matrix protein 1 promotes cell metastasis and glucose metabolism by inducing integrin β4/FAK/SOX2/HIF-1α signaling pathway in gastric cancer. *Oncogene* **37**, 744–755 (2018).
9. Z. Li *et al*., ECM1 controls T(H)2 cell egress from lymph nodes through re-expression of S1P(1). *Nat. Immunol.* **12**, 178–185 (2011).
10. P. Su *et al*., Novel function of extracellular matrix protein 1 in suppressing Th17 cell development in experimental autoimmune encephalomyelitis. *J. Immunol.* **197**, 1054–1064 (2016).
11. L. He *et al*., Extracellular matrix protein 1 promotes follicular helper T cell differentiation and antibody production. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 8621–8626 (2018).
12. W. Fan *et al*., ECM1 prevents activation of transforming growth factor beta, hepatic stellate cells, and fibrogenesis in mice. *Gastroenterology* **157**, 1352–1367.e13 (2019).
13. M. Dougan, G. Dranoff, S. K. Dougan, GM-CSF, IL-3, and IL-5 family of cytokines: Regulators of inflammation. *Immunity* **50**, 796–811 (2019).
14. A. Deiß, I. Brecht, A. Haarmann, M. Buttmann, Treating multiple sclerosis with monoclonal antibodies: A 2013 update. *Expert Rev. Neurother.* **13**, 313–335 (2013).
15. L. Egea, Y. Hirata, M. F. Kagnoff, GM-CSF: A role in immune and inflammatory reactions in the intestine. *Expert Rev. Gastroenterol. Hepatol.* **4**, 723–731 (2010).
16. Y. Xu, N. H. Hunt, S. Bao, The role of granulocyte macrophage-colony-stimulating factor in acute intestinal inflammation. *Cell Res.* **18**, 1220–1229 (2008).
17. S. K. Sainathan *et al*., Granulocyte macrophage colony-stimulating factor ameliorates DSS-induced experimental colitis. *Inflamm. Bowel Dis.* **14**, 88–99 (2008).
18. J. R. Korzenik, B. K. Dieckgraefe, J. F. Valentine, D. F. Hausman, M. J. Gilbert; Sargramostim in Crohn's Disease Study Group, Sargramostim for active Crohn's disease. *N. Engl. J. Med.* **352**, 2193–2201 (2005).
19. J. B. Eggesbø, I. Hjermann, G. B. Joø, R. Ovstebø, P. Kierulf, LPS-induced release of EGF, GM-CSF, GRO alpha, LIF, MIP-1 alpha and PDGF-AB in PBMC from persons with high or low levels of HDL lipoprotein. *Cytokine* **7**, 562–567 (1995).
20. P. Y. Berclaz *et al*., GM-CSF regulates a PU.1-dependent transcriptional program determining the pulmonary response to LPS. *Am. J. Respir. Cell Mol. Biol.* **36**, 114–121 (2007).
21. K. Yamaoka *et al*., Activation of STAT5 by lipopolysaccharide through granulocyte-macrophage colony-stimulating factor production in human monocytes. *J. Immunol.* **160**, 838–845 (1998).
22. G. Wu, S. M. Morris, Jr, Arginine metabolism: Nitric oxide and beyond. *Biochem. J.* **336**, 1–17 (1998).
23. A. P. Gobert *et al*., Protective role of arginase in a mouse model of colitis. *J. Immunol.* **173**, 2109–2117 (2004).
24. L. A. Coburn *et al*., L-arginine availability and metabolism is altered in ulcerative colitis. *Inflamm. Bowel Dis.* **22**, 1847–1858 (2016).
25. L. A. Coburn *et al*., L-arginine supplementation improves responses to injury and inflammation in dextran sulfate sodium colitis. *PLoS One* **7**, e33546 (2012).
26. J. I. Odegaard, A. Chawla, Alternative macrophage activation and metabolism. *Annu. Rev. Pathol.* **6**, 275–297 (2011).
27. R. Rutschman *et al*., Cutting edge: Stat6-dependent substrate depletion regulates nitric oxide production. *J. Immunol.* **166**, 2173–2177 (2001).
28. M. F. Neurath, Cytokines in inflammatory bowel disease. *Nat. Rev. Immunol.* **14**, 329–342 (2014).
29. S. Danese, C. Fiocchi, Ulcerative colitis. *N. Engl. J. Med.* **365**, 1713–1725 (2011).
30. C. Abraham, J. H. Cho, Inflammatory bowel disease. *N. Engl. J. Med.* **361**, 2066–2078 (2009).
31. C. G. Mayne, C. B. Williams, Induced and natural regulatory T cells in the development of inflammatory bowel disease. *Inflamm. Bowel Dis.* **19**, 1772–1788 (2013).
32. E. G. Perdiguero, F. Geissmann, The development and maintenance of resident macrophages. *Nat. Immunol.* **17**, 2–8 (2016).
33. S. Yona *et al*., Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* **38**, 79–91 (2013).
34. S. K. Biswas, A. Mantovani, Orchestration of metabolism by macrophages. *Cell Metab.* **15**, 432–437 (2012).
35. A. M. Kabat, E. J. Pearce, Inflammation by way of macrophage metabolism. *Science* **356**, 488–489 (2017).
36. D. R. Herbert *et al*., Arginase I suppresses IL-12/IL-23p40-driven intestinal inflammation during acute schistosomiasis. *J. Immunol.* **184**, 6438–6446 (2010).
37. S. Galván-Peña, L. A. O'Neill, Metabolic reprograming in macrophage polarization. *Front. Immunol.* **5**, 420 (2014).
38. T. Weinhage *et al*., Granulocyte macrophage colony-stimulating factor-activated CD39(+)/CD73(+) murine monocytes modulate intestinal inflammation via induction of regulatory T cells. *Cell. Mol. Gastroenterol. Hepatol.* **1**, 433–449.e1 (2015).
39. A. Suzumura, M. Sawada, T. Marunouchi, Selective induction of interleukin-6 in mouse microglia by granulocyte-macrophage colony-stimulating factor. *Brain Res.* **713**, 192–198 (1996).
40. X. Han *et al*., Tumour necrosis factor alpha blockade induces an anti-inflammatory growth hormone signalling pathway in experimental colitis. *Gut* **56**, 73–81 (2007).
41. L. M. DiFedele *et al*., Tumor necrosis factor alpha blockade restores growth hormone signaling in murine colitis. *Gastroenterology* **128**, 1278–1291 (2005).
42. S. Gilbert *et al*., Enterocyte STAT5 promotes mucosal wound healing via suppression of myosin light chain kinase-mediated loss of barrier function and inflammation. *EMBO Mol. Med.* **4**, 109–124 (2012).
43. W. Sheng *et al*., STAT5 programs a distinct subset of GM-CSF-producing T helper cells that is essential for autoimmune neuroinflammation. *Cell Res.* **24**, 1387–1402 (2014).
44. Y. Zhang *et al*., Activation of vascular endothelial growth factor receptor-3 in macrophages restrains TLR4-NF-κB signaling and protects against endotoxin shock. *Immunity* **40**, 501–514 (2014).
45. National Research Council, *Guide for the Care and Use of Laboratory Animals* (National Academies Press, Washington, DC, ed. 8, 2011).